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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

UNIVERSITY OF LEICESTER
DEPT. OF MEDICINE & THERAPEUTICS
CLINICAL SCIENCES BUILDING
LEICESTER, LE2 7LX

Patents ADP number *(if you know it)* 07983480003

If the applicant is a corporate body, give the country/state of its incorporation

UK

4. Title of the invention

METHOD FOR PREDICTION OF CARDIAC DISEASE

5. Name of your agent *(if you have one)*

ANDREWS, MARTYN P

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

PATENT DEPT, UNIPATH LTD
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BEDFORD, MK44 3UP

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 22.10.02 (22ND OCTOBER 200

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Field of the Invention

This invention relates to the use and measurement of cardiac biomarkers and additional cofactors in the screening of patients for left ventricular systolic dysfunction (LVSD). In particular it relates to the use of cardiac biomarkers in combination with electrocardiogram (ECG) and history of ischaemic heart disease and optionally other risk factors indicative of cardiac disease. This invention also relates to an algorithm in order to interrogate the patient's natriuretic peptide level in combination with, in particular major abnormalities found in the patient's ECG data in order to obtain an improved indication of the likelihood of a patient either having or not having LVSD.

Background to the invention

Heart failure is a chronic, progressive disease that affects 1.5-2% of the general population of the Western World. Clinically, the term 'heart failure' is applied to the syndrome of breathlessness and fatigue, often accompanied by fluid retention, as indicated by an elevated jugular venous pressure and oedema. In persons over the age of 65 years, the incidence increases to 6-10%. Heart failure is the most frequent cause of hospitalisation in elderly patients and is recognised as a major health problem. In the USA, 4.6 million individuals have a diagnosis of heart failure and a further 400,000 to 700,000 patients are diagnosed each year, costing the healthcare system nearly $38 billion for in-patient ($23.1 billion) and out-patient care ($14.7 billion) each year. In particular, hospital admission and readmissions account for the majority of this expenditure. Latest findings estimate that as many as 20m people with heart failure in the USA are undiagnosed.

Heart failure is most commonly due to LVSD where the myocardium fails to contract normally and the left ventricle is usually dilated. Previous acute myocardial infarction (AMI), chronic hypertension, dilated cardiomyopathy, viral myocarditis, Chagas' disease and alcoholic heart disease are common causes of myocardial systolic failure.

Patients with heart failure are categorised into different risk groups according to the New York Heart Association (NYHA) functional classification system. This system relates symptoms to everyday activities and the patient's quality of life. The system has four classes. In Class I, patients have cardiac disease but without the resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain. In Class II, patients have cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain. In Class III, patients have cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain. Finally, in Class IV, patients have cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

It will be clear that individuals with Class I heart failure and some patients with Class II heart failure cannot easily be identified from patients without heart failure in the general population using clinical history alone. Therefore, in a group of apparently healthy individuals who do not have any presenting symptoms or obvious recent symptoms of heart failure, identifying these patients using the NYHA criteria for further investigation is not

possible. Even if the patient had symptoms suggestive of heart failure, these symptoms overlap with many other conditions and on their own are not specific for heart failure.

Because early stages of heart failure go undetected, diagnosis of heart failure often only occurs when the patient's condition is at a more advanced stage, for example, following presentation to the hospital with acute decompensation. However, it is known that a proportion of apparently healthy individuals will indeed have LVSD. Further, it is known that these patients if treated will benefit from slowed disease progression, fewer hospital re-admissions, and an improved quality of life.

The definitive method to diagnose heart failure is echocardiography. The echocardiogram provides an accurate means to diagnose LVSD and hence heart failure. However, echocardiogram is a skilled technique requiring expertise and is not available to the generalist physician. Further, echocardiography is relatively expensive and access to echocardiography facilities for the generalist physician is frequently inadequate. In routine practice, therefore, generalist physicians rely on clinical features to make a presumptive diagnosis of heart failure, a strategy known to be inaccurate.

Other tests are available to the generalist physician that might have a role in identifying previously undiagnosed patients with LVSD. Studies have evaluated the use of electrocardiography (ECG) and natriuretic peptides.

The resting ECG is the most widely used cardiovascular diagnostic test. Currently, approximately one half of all ECGs are performed by physicians without special training in cardiology. The value of any screening test depends critically on four key principles: its cost; the prevalence of the abnormalities detected in the population assessed; the relationship of the abnormalities to morbidity and mortality; and the possibility of reducing or avoiding future morbidity or mortality given the information provided by the test. In particular, to be worth the additional expense, the ECG must add significantly to the ability of standard risk factors to identify previously undiagnosed individuals with sub-clinical disease. The validity of using the resting 12-lead electrocardiogram as a screening test for cardiovascular disease in apparently healthy individuals has never been convincingly demonstrated.

While some studies have suggested that a significant proportion of patients with LVSD have a normal ECG, others have concluded that the ECG is unlikely to be normal in the patient with LVSD. A significant proportion of patients with LVSD have a normal ECG (Houghton et al: Int. J. Cardiol. 1997; 62: 31-36). Unsurprisingly, previous screening studies have reported a higher prevalence of ECG abnormalities in patients with LVSD than in those with preserved LV systolic function. However, the prevalence of ECG abnormalities in the general population, and in particular in those age groups at risk of LVSD, has been reported to be of the order of 40-75%.

Natriuretic peptides (e.g. atrial natriuretic peptide [ANP], B-type natriuretic peptide [BNP], and their respective prohormones, N-terminal proANP [NTproANP or N-ANP] and N-terminal proBNP [NTproBNP or N-BNP]) have been found to be elevated in patients with LVSD. Studies have focused on the diagnostic and prognostic usefulness of natriuretic peptide measurement. BNP in particular has been demonstrated to discriminate cardiac and non-cardiac dyspnea (i.e. breathlessness), provide prognostic data on future left ventricular function and survival when measured within the days following myocardial infarction, and allow patients with heart failure to be stratified into risk groups dependent on their BNP level.

BNP is a cardiac neurohormone secreted from the cardiac ventricles as a response to ventricular volume expansion and pressure overload. Levels of BNP are elevated in cardiac disease states associated with increased ventricular stretch. BNP levels are reflective of left ventricular diastolic filling pressures and thus correlate with pulmonary capillary wedge pressure. BNP levels have been shown to be elevated in patients with symptomatic left ventricular dysfunction and correlate with New York Heart Association (NYHA) classification and prognosis. Distinguishing congestive heart failure from other causes of dyspnea is of great importance in patients presenting for medical attention with signs and/or symptoms that may or may not represent heart failure. A number of studies have demonstrated the limited reliability of the physical examination and Chest X-ray in diagnosing heart failure. Even with the best of clinicians, diagnosing heart failure remains a clinical challenge. BNP measurements are now in routine use in the emergency department and urgent-care settings. This assay represents the first clinically available blood test to facilitate the diagnosis of heart failure in patients presenting with symptoms.

BNP can be measured using standard laboratory immunoassay methods (e.g. Shionogi SHIONORIA BNP). Further, a new point-of-care (POC) diagnostic for use in the emergency room or decentralised settings (e.g. heart failure clinic) is now available. The POC assay for BNP, known as the Triage BNP Test, is commercially available from Biosite Incorporated. The assay utilises a fluorescence detection system to measure BNP in whole blood within 15 minutes. . The result of the measurement is displayed as a concentration of BNP found in the patient's blood sample, reported in pg/ml. The lower limit of detection is 20 pg/ml and a diagnostic level to exclude heart failure is BNP < 100 pg/ml. A level of > 100 pg/ml is considered positive and indicative of heart failure.

Studies that have investigated the utility of the natriuretic peptides in population screening for LVSD have observed that the positive predictive value of natriuretic peptides for the presumptive diagnosis of heart failure is low. Thus detection of elevated levels of BNP is not necessarily indicative of the presence or likelihood of LVSD. Patients with lung cancer, pulmonary embolism, myocardial infarction, and end-stage renal disease can also have elevated levels. For this reason, measurement of BNP in a healthy population will identify a significant number of patients with elevated levels, many of whom will not have LVSD. The positive predictive value of the natriuretic peptides in screening unselected populations (i.e. the general population) has been demonstrated to be <20%. These studies have evaluated the predictive value of a natriuretic peptide value above one or more given concentrations. The conclusions made by the authors of these studies is that natriuretic peptide levels have a high negative predictive value (a 'normal' value effectively ruling out LVSD), but the positive predictive value is weak (an 'elevated' natriuretic peptide value does not necessarily mean that the patient has LVSD). Further, none of the studies have attempted to assess the specificity and positive predictive value of the natriuretic peptides at 100% sensitivity, an important requirement for an effective screening method. In practical terms, if a generalist physician used the results of natriuretic peptide measurements alone at a cut-off optimised to include all patients with LVSD, he will also rule-in a substantial number of patients without LVSD. Whilst it is accepted that measurement of natriuretic peptides is a useful tool in confirming that patients presenting with dyspnea in the acute setting have LVSD, use of natriuretic peptides on their own are of limited use in the identification or screening of patients with LVSD in the community. Similarily, as described above, the use of ECG measurement on its own is also of limited value.

Other biomarkers whose levels are known to be altered in patients with LVSD have been described in the literature. These include Endothelin-1, Big Endothelin-1, Adrenomedullin, Urotensin, Angiotensin II, Uroguanylin, and cell injury markers including troponin I and T. Similar to the natriuretic peptides, consideration of the level of these biomarkers does not enable a clear distinction between patients with or without LVSD.

There is no simple method that would enable a generalist physician to identify a group of patients with previously undiagnosed LVSD without including an unacceptable number of false-positive patients. Similarly, for an individual, there exists a need to be able to identify the existence of LVSD with a reasonable degree of confidence such that that patient is not sent unnecessarily for further investigation.

For the first time it is possible to cost-effectively screen normal apparently healthy individuals to identify patients with a high risk of LVSD. These patients can then be investigated further using echocardiography. The benefit to the patient is that their condition will be identified at an earlier stage (before they present with clinical symptoms) allowing appropriate effective treatment to be implemented. The benefit to society is that patients with heart failure will lead healthier lives for longer and will not consume the same level of resources as they would if they were first identified following hospital admission for, for example, an episode of acute decompensation. There exists therefore a need for an improved means of screening patients in order to identify those with previously undiagnosed LVSD, thus enabling earlier intervention.

Summary of the invention

This invention overcomes the shortcomings of the above-mentioned prior art and provides a method for the screening of patients in order to identify a patient or a group of patients in whom the probability of LVSD is high.

The invention also concerns an algorithm in order to process the data obtained from both the ECG and natriuretic peptide measurements such as to give an indication of the likelihood of a patient having LVSD. Importantly, the use of the algorithm is essential for this result to be obtained. In other words, the same result could not be achieved merely from the consideration of the particular natriuretic peptide concentration in combination with study of the particular ECG traces. The invention as described herein refers to the measurement of a natriuretic peptide. However, it will be obvious that an alternative biomarker such as one of those listed above could be used to construct the algorithm using the methods described.

According to one embodiment, the invention also provides for a device for measuring BNP and/or ECG. The device for measuring natriuretic peptides and/or ECG would either have the algorithm contained within the device by incorporation into software or have the means to receive the result as calculated by the algorithm remotely from the device. Additionally a device to measure either natriuretic peptides or ECG alone would have the means to accept respectively the results obtained from the ECG and natriuretic peptide measurements. Alternatively, the algorithm could reside on a computer and the user input the data obtained from both the natriuretic peptide measurement and the ECG data.

The objectives of the invention have been achieved by consideration of various cofactors in combination with natriuretic peptide measurements. In particular these cofactors

relate to data obtained from ECG measurements and considerations of a previous history of myocardial infarction (MI) or angina. In particular, these cofactors concern a major ECG abnormality (i.e. Q-wave, left-bundle branch block, left ventricular hypertrophy or atrial fibrillation) and a history of MI or angina. Moreover, the predictive value of the model is weakened only minimally by consideration of a natriuretic peptide and the ECG alone. Addition of the ECG result reduces the number of patients who would require an echocardiogram based solely on a natriuretic peptide level by four-fold.

As expected, the measurement of a natriuretic peptide alone, for example BNP, resulted in an unacceptably low positive predictive value of 2.24%. Also, in agreement with previous studies, we found that a high prevalence of ECG abnormalities in the general population (24% had a minor and 16% a major ECG abnormality). Therefore, as a screening tool, ECG suffered from a lack of sensitivity and specificity for the prediction of LVSD.

Interpretation of natriuretic peptide measurement and ECG together increased specificity of the test significantly without any loss of sensitivity (retained at 100%). In terms of screening a low risk population, randomly selected from primary care and without a prior diagnosis of heart failure, all patients with the condition can be identified while minimising the number of patients unnecessarily requiring echocardiographic examination.

We have defined an algorithm employing the patient's natriuretic peptide level alongside additional cofactors, in particular major abnormalities found in the patient's ECG. The algorithm identifies all patients with LVSD and a substantially reduced number of false-positives. This provides for the first time a method that can be used to cost-effectively screen patients for previously undiagnosed heart failure. The improved specificity achievable at 100% sensitivity results in fewer subjects from the population needing to be investigated further by echocardiography scans.

Detailed description of the invention

Thirteeen hundred and thirty nine patients (men aged 45-80 and women aged 55-80) of 2392 patients who were approached were entered into the study. All selected patients were from primary care and without a prior diagnosis of heart failure or LVSD. Demographic features are shown in Table 1. Information collected included past medical history of ischaemic heart disease (myocardial infarction or angina), hypertension, diabetes, smoking status, information on prescribed medication, and a check that the patient had not had a confirmed prior diagnosis of heart failure or LVSD. The criterion standard used to diagnose LVSD was echocardiography performed using a Sonos 5500 machine (Philips Technologies). Wall motion score indices (where a score of >2 is indicative of hypokinesis, akinesis, or dyskinesis) and ejection fractions measured during echocardiography were obtained using recognised methods.

Twenty mls of peripheral venous blood was drawn into pre-chilled Na-EDTA (1.5mg/ml blood) tubes containing 500 IU/ml aprotinin. After centrifugation at 3000 rpm at 4°C for 15 min, plasma was separated and stored at –70 °C until assay.

Prior to assay of N-ANP and BNP, plasma was extracted on C_{18} Sep-Pak (Waters) columns and dried on a centrifugal evaporator. Assays for N-ANP and BNP were based on commercially available antibodies from Peninsular Laboratories Inc (Belmont, CA, USA)

and Phoenix Pharmaceuticals Inc.(Belmont, CA, USA) respectively. The tracer peptides were biotinylated using biotin-X-N-hydroxysuccinimide ester (Calbiochem, Nottingham, UK) and purified on reverse phase C_{18} HPLC using an acetonitrile gradient. Plasma extracts and standards were reconstituted with ILMA (immunoluminometric assay) buffer consisting of (in mmol/l) NaH_2PO_4 1.5, Na_2HPO_4 8, NaCl 140, EDTA 1 and (in g/l) bovine serum albumin 1, azide 0.1. ELISA plates were coated with 100 ng of anti-rabbit IgG (Sigma Chemical Co., Poole, UK) in 100 µl of 0.1 mol/l sodium bicarbonate buffer, pH 9.6. A competitive immunoluminometric assay was set up by preincubating 50 ng of the anti N-ANP or BNP IgG with standards or samples within the wells. After overnight incubation, 50 µl of the diluted biotinylated N-ANP or BNP peptide tracer (1 µl /ml of the stock solution) was added to the wells. Following another 24 h of incubation at 4°C, wells were washed 3 times. Streptavidin labeled with methyl-acridinium ester was used to detect the tracer bound in the wells. The lower limits of detection of N-ANP and BNP were 3.4 and 2.0 pM respectively. There was no cross reactivity between these assays.

Unextracted plasma was assayed for N-BNP using a non-competitive immunoluminometric assay which was based on the non-competitive N-terminal proBNP assay described by Karl (Development of a novel, N-terminal-proBNP (NT-proBNP) assay with a low detection limit. Scand J Clin Lab Invest Suppl 1999;230:177-181). Rabbit polyclonal antibodies were raised to the N-terminal (amino acids 1-12) and C-terminal (amino acids 65-76) of the human N-terminal proBNP.

IgG from the sera was purified on protein A sepharose columns. The C-terminal directed antibody (0.5 µg in 100 µL for each ELISA plate well) served as the capture antibody. The N-terminal antibody was affinity purified and biotinylated. Aliquots (20µL) of samples or N-BNP standards were incubated in the C-terminal antibody coated wells with the biotinylated antibody for 24 hours at 4°C. Following washes, streptavidin labeled with methyl-acridinium ester was used to detect bound biotinylated antibody. The lower limit of detection was 5.7 pM of unextracted plasma. There was no cross-reactivity with ANP, N-ANP, BNP or CNP.

Twelve-lead ECGs were analysed for the presence of major (pathological Q wave, left bundle branch block, left ventricular hypertrophy, atrial flutter/fibrillation) and minor (left axis deviation, right bundle branch block, poor R-wave progression, atrial hypertrophy, non-specific ST segment change, sinus bradycardia or tachycardia) abnormality.

Statistical analysis was performed using the SPSS package (version 11.0, SPSS Inc, IL). Natriuretic peptide levels were normalised by log transformation before analysis. Data was investigated using both parametric and non-parametric analysis of variance to identify which factors and covariates relate to LVSD. Logistic regression analysis was performed for predicting the presence of LVSD with factors and covariates which were related to LVSD in univariate analysis and the probability of membership of either group (the prognostic index) saved for plotting of Receiver Operating Characteristic (ROC) curves. This logistic regression analysis can be performed using different statistical software packages (of which SPSS is an example), and yields an equation for predicting the $\log_e$ of the odds ratio (defined as the ratio of the probability of having LVSD to the probability of not having LVSD), the equation having terms such as a constant and coefficients defined as B_1 to B_n (n referring to the number of predictor variables in the equation) by which the different predictor variables are multiplied. When these different terms are added together, the $\log_e$ of the odds ratio can be determined.

The diagnostic accuracy of different computations of variables is compared with a ROC curve. The ROC curve displays the relationship between the sensitivity and specificity of a test at different test cut-off levels. In the case of screening patients for LVSD, the area under the ROC curve indicates how well the test can separate patients with and without LVSD. An ideal test would have an area under the curve of 1.0 meaning that both sensitivity and specificity of the test is 100 per cent. A test that could not distinguish patients with and without LVSD would have an area under the curve of 0.5. ROC curves can be used to compare the effectiveness of the measurement of an individual biomarker to, alternatively, the effectiveness of a combination of variables including, for example, presence or absence of abnormalities in an ECG trace, the result of a measurement of one or more biomarkers, and the presence or absence of a medical history of myocardial infarction or angina. For a combination of variables, it is necessary to determine by computation, a prognostic index from an algorithm that delivers the highest specificity at 100% sensitivity and hence the best ability to identify with confidence a patient with LVSD.

17 individuals (1.3%) had significant LVSD based on the left ventricular wall motion index (LVWMI) score of ≥ 2 (equivalent of an ejection fraction of <35%, representing severe heart failure that will benefit from institution of therapy such as angiotensin converting enzyme inhibitors and certain beta blockers). Plasma concentration of each natriuretic peptide was higher in those with LVSD than in those with preserved systolic function: median N-ANP (range) 943.4 (288.4 – 3020) pM *vs* 385.0 (5.2-4115.4) pM ($p<0.0005$); BNP 92.9 (19.0-501.2) pM *vs* 17.1 (2.0-275.4) pM ($p<0.0005$); N-BNP 301.6 (38.0-1230.3) pM vs 36.3 (5.8-1174.9) pM, ($p<0.0005$).

Comparison of the areas under the ROC curves for N-ANP (0.810) BNP (0.943), and N-BNP (0.871) showed BNP to be superior in the detection of LVSD (Figure 1). At 100% sensitivity for the detection of LVWMI ≥ 2, the specificity of N-ANP (287 pM), BNP (19.2 pM) and N-BNP (37.6 pM) was 27%, 47% and 47% respectively.

Data was further analysed by applying the standard statistical analysis method, logistic regression. Logistic regression involves fitting to the data an equation of the form '$\text{logit}(p) = a + b_1x_1 + b_2x_2 + b_3x_3 + \ldots$', where $\text{logit}(p) = \log_e (p/(1-p))$.

In a logistic regression analysis for univariate determinants of LVWMI ≥ 2, potential factors or covariates included were $\log_{10}$ plasma natriuretic peptide, age, gender, plasma creatinine, major ECG abnormality, minor ECG abnormality, body mass index, and past history of MI or angina, of diabetes or of hypertension. In a multivariate logistic regression analysis (Table 2), plasma BNP was the strongest predictor of LVSD ($p<0.0005$), the only other factors retaining independent predictive value being major ECG abnormality ($p=0.006$) and a history of MI or angina ($p=0.029$).

A decision of whether there are any major abnormalities in the patient's ECG trace leads to a weighting of, for example, of either '0' or '1'. '0' means there were no major abnormalities in the ECG trace and '1' means that an abnormality was detected. The identification of an abnormality, for example, indicative of left ventricular hypertrophy (LVH), left-bundle branch block (LBBB), atrial fibrillation, or the presence of a Q-wave, is easily identified by visual observation of the ECG trace or alternatively, can be determined using appropriate software such as the GE 12SL ECG analysis computer program from GE Medical Systems. This software makes precise measurements of recorded cardiac signals,

then provides an interpretation of the ECG waveforms using classic and newly developed ECG interpretation criteria for both rhythm and morphology.

The ROC curves for BNP alone and for the prognostic index derived from the combination of BNP, the ECG and a history of MI or angina are shown in Figure 2. The area under the ROC curve increased from 0.94 with BNP alone, to 0.978 with the regression model. It is clear that the specificity of the model was markedly improved compared to that of BNP alone (Figure 2, Table 3). When the model contained only the two strongest predictors, BNP and major ECG abnormality, the area under the curve was 0.974. Once again specificity of this model was clearly superior to that of BNP alone (Figure 2).

When the regression analysis was repeated for the prediction of LVEF $\leq$ 35% rather than for LVWMI, very similar results were obtained. There were 16 individuals with LVEF $\leq$ 35% and the same factors emerged as predictive on multivariate analysis. The odds ratio for a 50% increase in plasma BNP (2.41) was almost identical to that for prediction of LVWMI $\geq$ 2 (2.43). However the area under the ROC curve and the specificity of the model when sensitivity was set at 100% were slightly less (Table 3).

The ECG alone was neither sensitive nor specific for the identification of LVWMI $\geq$ 2. Of the 17 individuals with LVWMI $\geq$ 2, the ECG was normal in 2 (12%). Thus the ECG alone could not attain 100% sensitivity for detection of LVSD in our population. The finding of 1 or more minor ECG abnormalities had a specificity of 60.7% and PPV of 3.1% for both LVWMI $\geq$ 2 and LVEF $\leq$ 35%.

The coefficients for the full logistic model for predicting LVWMI $\geq$ 2 (constant term, B_1, B_2 and B_3) are presented in Table 4. The $\log_{10}$ BNP level is expressed in pM, presence or absence of a major ECG abnormality is coded as 1 or 0, and presence or absence of an ischaemic heart disease history (MI or angina) coded as 1 or 0. The equations for the full model take the general form :

$$\text{Log}_e\ p/(1-p) = \text{Constant} + B_1*(\log_{10}\text{BNP}) + B_2*(\text{ECG abnormality, 1 or 0}) + B_3*(\text{history of MI or angina, 1 or 0})$$

where p is the probability of having heart failure as defined by LVWMI $\geq$ 2.

The coefficients for the minimal logistic model for predicting LVWMI $\geq$ 2 (constant term, B_1 and B_2) are presented in Table 5. The equations for the minimal model take the general form :

$$\text{Log}_e\ p/(1-p) = \text{Constant} + B_1*(\log_{10}\text{BNP}) + B_2*(\text{ECG abnormality, 1 or 0})$$

Logistic regression analysis was performed to predict LVSD as defined by LVWMI $\geq$2 using either N-ANP or N-BNP levels, and factors such as major ECG abnormality and history of MI or angina. These models and the coefficients for the peptide levels, ECG findings or ischaemic history are presented in Tables 4 and 5. For both the full model (peptides, ECG, ischaemic history) and the minimal model (peptides, ECG), the ROC areas for N-ANP and N-BNP were markedly improved (Tables 4, 5, Figure 3). In addition, the

specificities for diagnosis of heart failure (at 100 % sensitivity) were also greatly improved when compared to use of the peptides alone (Table 6).

This resulted in increased positive predictive values for both the full and the minimal model for all the peptides studied. This improved specificity also resulted in less subjects from the population having a "positive" test, hence markedly reducing the number of screenees that need to be investigated further by echocardiography scans (Table 6).

The use of these logistic equations improves markedly the accuracy of all the natriuretic peptides studied (N-ANP, N-BNP and BNP), resulting in increased specificity of detection of heart failure (at 100% sensitivity), increased positive predictive values and hence the number of false positive tests.

The model can be made to be applicable to different centres where normal ranges may differ, by ranking all the peptide levels and expressing them as percentiles. The percentiles are then entered into logistic regression analysis, with presence or absence of major ECG abnormalities and/or presence or absence of history of MI or angina as factors. The models are then as follows:

Full model:

$$\text{Log}_e\ p/(1-p) = \text{Constant} + B_1*(\text{peptide centile, as \%}) + B_2*(\text{ECG abnormality, 1 or 0}) + B_3*(\text{history of MI or angina, 1 or 0})$$

Minimal model:

$$\text{Log}_e\ p/(1-p) = \text{Constant} + B_1*(\text{peptide centile, as \%}) + B_2*(\text{ECG abnormality, 1 or 0})$$

where p is the probability of having heart failure as defined by LVWMI ≥ 2.

The coefficients for the full logistic models (with peptides, ECG, history of MI or angina) or minimal models (with peptides and ECG) are presented in Tables 7 and 8. The resulting ROC curves plotted from the derived prognostic indices of the models all had greater underlying areas than the ROC curves plotted with peptide data alone (Tables 7, 8 and figure 4). The specificities of the logistic models in achieving a diagnosis at 100 % sensitivity are presented in Table 9. Both full and minimal models with all peptides had improved specificities compared to use of the peptide data alone in diagnosis of heart failure (Tables 7,8, figure 4). Thus, use of the coefficients reported in Tables 7 and 8 allow the calculation of a prognostic index based on the centile of the peptide used, this having similar utility to the absolute level of the peptide.

Given the similar diagnostic accuracy of other biomarkers (for example, Endothelin-1, Big Endothelin-1, Adrenomedullin, Urotensin, Angiotensin II, and Uroguanylin) one would expect the logistic model to work for these biomarkers following derivation of new constants B_1, B_2, and B_3. Therefore, one would expect that these biomarkers could also be used to identify patients with a high probability of LVSD.

Table 1 : Characteristics of study population (n = 1339)

Men/Women: (number (%))	756 : 583 (56.40/43.6)
Age (years): mean (range)	63 (45 – 81)
Practice Jarman score: mean (range)	+7.1 (-16.0 -to + 41.4)
BMI: mean ±SD	26.7 ± 4.4
Systolic blood pressure: mean ±SD	135 19)
Diastolic blood pressure: mean (SD)	78 ± 12
Current smoker: number (%)	261 (19.6)
Body Mass Index (kg/m^2)	28.5 ± 4.5
Medical History (n (%))	
Myocardial infarction	33 (2.5)
Angina	92 (6.9)
Hypertension	322 (24)
Diabetes mellitus	63 (4.7)
Prescribed therapy	
ACE inhibitor/ ARA	117 (8.8)
Loop diuretic	36 (2.7)
Other diuretic	171 (12.8)
Beta-blocker	151 (11.3)
Nitrate	53 (4)
Calcium channel blocker	134 (10)
Digoxin	9 (0.7)
Natriuretic peptides (pM: median (range)	
N-ANP	401.1 (5.2-4115.4)
BNP	20.3 (2-507.9)
N-BNP	44.8 (5.7-1230.2)

Abbreviations: SD: standard deviation; BMI: body mass index; ACE: angiotensin-converting enzyme; ARA: angiotensin-II receptor antagonist.

Table 2: Multivariate analysis for determinants of LVWMI ≥ 2

Factor	Odds Ratio	p
Gender (Male)	1.2	0.809
Creatinine	1	0.443
Major ECG abnormality	9.8	0.006
BNP*	2.4	<0.0005
History of MI or angina	3.9	0.029

* Odds ratio for 50% increase in BNP

Table 3

Sensitivity and areas under the ROC curve of BNP, the prognostic indices derived for the full regression model and the minimal regression model, for the prediction of LVWMI score ≥ 2 and for the prediction of LVEF ≤ 35%. The full regression model contains BNP+ECG+ history of MI/angina; the minimal regression model contains BNP+ECG. The quoted specificities are for a sensitivity of 100%.

	Left Ventricular Wall Motion Index Score ≥ 2		Left Ventricular Ejection Fraction ≤ 35%	
	AUC	Specificity %	AUC	Specificity %
BNP	0.943	47	0.943	46
BNP+ECG+ history of MI/angina	0.978	92	0.973	88
BNP+ECG	0.974	88	0.973	88

Table 4.
Coefficients for the covariates (peptides) and factors for the full model for diagnosis of heart failure, as defined by a LVMI > 2.0 . SEMs of the coefficients are in brackets. Areas under the ROC curves were obtained from the predicted probability odds ratios using the model coefficients.

	Log_{10} Peptide (pM) B_1	ECG B_2	PMH of angina or MI B_3	Constant	Nagelkerke r^2	ROC area
BNP	4.681 (0.996)	2.249 (0.831)	1.352 (0.615)	-13.230 (1.762)	0.517	0.978 (0.007)
Odds Ratio	107.919	9.48	3.864			
N-ANP	2.951 (0.954)	2.936 (0.788)	1.785 (0.539)	-14.533 (2.751)	0.393	0.951 (0.017)
Odds Ratio	19.13	18.844	5.959			
N-BNP	2.243 (0.706)	2.716 (0.791)	1.515 (0.543)	-10.856 (1.715)	0.416	0.956 (0.014)
Odds Ratio	9.421	15.115	4.547			

Table 5.
Coefficients for the covariates (peptides) and factors for the minimal model for diagnosis of heart failure, as defined by a LVMI > 2.0 . SEMs of the coefficients are in brackets. Areas under the ROC curves were obtained from the predicted probability odds ratios using the model coefficients.

	Log_{10} Peptide (pM) B_1	ECG B_2	Constant	Nagelkerke r^2	ROC area
BNP	4.996 (0.946)	2.525 (0.806)	-13.561 (1.724)	0.492	0.974 (0.008)
Odds Ratio	147.818	12.496			
N-ANP	3.135 (0.879)	3.172 (0.781)	-14.692 (2.536)	0.335	0.939 (0.014)
Odds Ratio	22.98	23.854			
N-BNP	2.408 (0.648)	2.949 (0.779)	-10.929 (1.611)	0.374	0.974 (0.008)
Odds Ratio	11.113	19.083			

Table 6.
All figures refer to 100 % sensitivity for detection of heart failure in the screening population. The specificities, positive predictive values and % of the screenees that need to be investigated by scanning (due to a positive test result) are presented, for diagnosis of heart failure by the peptides (individually) alone, in combination with the ECG major abnormalities and ischaemic heart disease history (full model) or in combination with ECG major abnormalities (minimal model). The full and minimal models employed $\log_{10}$ peptide values in the model.

		Peptide only	Full model	Minimal model
BNP	Specificity %	47	92	88
	Positive Predictive value %	2.4	16.2	10.8
	% of screenees necessary to scan	52	8	12
N-ANP	Specificity %	27	76	80
	Positive Predictive value %	1.8	5.4	6.5
	% of screenees necessary to scan	72	24	20
N-BNP	Specificity %	47	82	85
	Positive Predictive value %	2.4	7.2	8.6
	% of screenees necessary to scan	52	18	15

Table 7.
Coefficients for the covariates (peptide levels expressed as rank percentile) and factors for the full model for diagnosis of heart failure, as defined by a LVMI > 2.0. SEMs of the coefficients are in brackets. Areas under the ROC curves were obtained from the predicted probability odds ratios using the model coefficients.

	Peptide ranked as centile %	ECG	PMH of angina or MI	Constant	Nagelkerke r^2	ROC area
BNP	0.101 (0.035)	2.44 (0.804)	1.299 (0.563)	-14.015 (3.153)	0.478	0.969 (0.013)
Odds Ratio	1.106	11.467	3.665			
N-ANP	0.026 (0.012)	3.121 (0.777)	1.745 (0.537)	-8.177 (1.099)	0.367	0.949 (0.017)
Odds Ratio	1.027	22.676	5.724			
N-BNP	0.044 (0.017)	2.878 (0.784)	1.482 (0.545)	-9.394 (1.482)	0.392	0.954 (0.014)
Odds Ratio	1.045	17.774	4.400			

Table 8.
Coefficients for the covariates (peptide levels expressed as rank percentile) and factors for the minimal model for diagnosis of heart failure, as defined by a LVMI > 2.0. SEMs of the coefficients are in brackets. Areas under the ROC curves were obtained from the predicted probability odds ratios using the model coefficients.

	Log_{10} Peptide (pM)	ECG	Constant	Nagelkerke r^2	ROC area
BNP	0.114 (0.036)	2.647 (0.788)	-14.913 (3.261)	0.45	0.962 (0.019)
Odds Ratio	1.12	14.11			
N-ANP	0.032 (0.012)	3.324 (0.77)	-8.251 (1.095)	0.311	0.938 (0.015)
Odds Ratio	1.033	27.783			
N-BNP	0.053 (0.017)	3.067 (0.774)	-9.749 (1.50)	0.352	0.952 (0.012)
Odds Ratio	11.113	19.083			

Table 9.

All figures refer to 100 % sensitivity for detection of heart failure in the screening population. The specificities, positive predictive values and % of the screenees that need to be investigated by scanning (due to a positive test result) are presented, for diagnosis of heart failure by the peptides (individually) alone, in combination with the ECG major abnormalities and ischaemic heart disease history (full model) or in combination with ECG major abnormalities (minimal model). The full and minimal models employed peptide values ranked as percentiles in the model.

		Peptide only	Full model	Minimal model
BNP	Specificity %	47	79	66
	Positive Predictive value %	2.4	6.2	3.8
	% of screenees necessary to scan	52	21	34
N-ANP	Specificity %	27	76	80
	Positive Predictive value %	1.8	5.4	6.5
	% of screenees necessary to scan	72	24	20
N-BNP	Specificity %	47	82	86
	Positive Predictive value %	2.4	7.2	9.2
	% of screenees necessary to scan	52	18	14

Claims

1. A method for screening an individual or group of patients for the likelihood of having LVSD comprising, in any order of (a), (b) or (c);

(a) measurement of the levels of a biomarker in a sample or samples of bodily fluid of said patient;
(b) conducting an ECG measurement on said patient or group of individuals; identification of the presence or absence of one or more major abnormality factors from the ECG trace; and optionally
(c) identification of the presence or absence of one or more cofactors which are known to be risk factors for CVD;

assigning or calculating weighting factors for (a), (b) and optionally (c);

obtaining a result indicative of the probability of said individual having LVSD.

2. A method according to claim 1 wherein the weighting factors for (a), (b) and optionally (c) are derived by logistic regression analysis on measurements of a biomarker, ECG findings, and of one or more cofactors which are known to be risk factors for CVD; wherein the patient population is taken from the general population and individuals have no previous diagnosis of LVSD.

3. A method according to claims 1 and 2 wherein the biomarker is a natriuretic peptide

4. A method according to claims 1 and 2 wherein one or more cofactors are selected from MI and angina.

5. An algorithm for the determination of the likelihood of an individual of having LVSD according to the following formula:

$$\text{Log}_e\ p/(1-p) = \text{Constant} + B_1*(y) + B_2*(\text{ECG abnormality, a}) + B_3*(\text{history of MI or angina, a})$$

where p is the probability of having heart failure as defined by LVSD;

B_1, B_2, and B_3 are the coefficients for the logistic model for predicting LVSD;

Wherein 'a' is a factor to indicate the presence or absence of ECG abnormality and history of MI or angina and wherein 'a' refers to any two numbers sufficiently separated as to impart a different weighting on the coefficients B_2 and B_3 in the presence or absence of ECG abnormality and history of MI or angina.

'y' is either $\log_{10}$ natriuretic peptide expressed in pM, or peptide centile;

wherein peptide centile, expressed as per cent, is determined by ranking all biomarker levels determined by measuring the biomarker level for an apparently healthy population using a chosen assay kit and expressing them as percentiles.

6. An algorithm for the determination of the likelihood of an individual of having LVSD according to the following formula:

$\text{Log}_e\ p/(1-p) = \text{Constant} + B_1*(y) + B_2*(\text{ECG abnormality, a})$

where p is the probability of having heart failure due to LVSD

B_1 and B_2 are the coefficients for the logistic model for predicting LVSD;

Wherein 'a' is a factor to indicate the presence or absence of ECG abnormality and history of MI or angina and wherein 'a' refers to any two numbers sufficiently separated as to impart a different weighting on the coefficient B_2 in the presence or absence of ECG abnormality and history of MI or angina.

'y' is either $\log_{10}$ natriuretic peptide expressed in pM, or peptide centile;

wherein peptide centile, expressed as per cent, is determined by ranking all biomarker levels determined by measuring the biomarker level for an apparently healthy population using a chosen assay kit and expressing them as percentiles.

7. A device for measurement of a biomarker and/or ECG incorporating either the algorithm or having processing means by which to specifically process the algorithm according to claims 5 and 6.

Figure 1.

ROC curves for the 3 peptides (N-ANP, BNP and N-BNP) in diagnosis of heart failure (as defined by a LVWMI score ≥ 2.

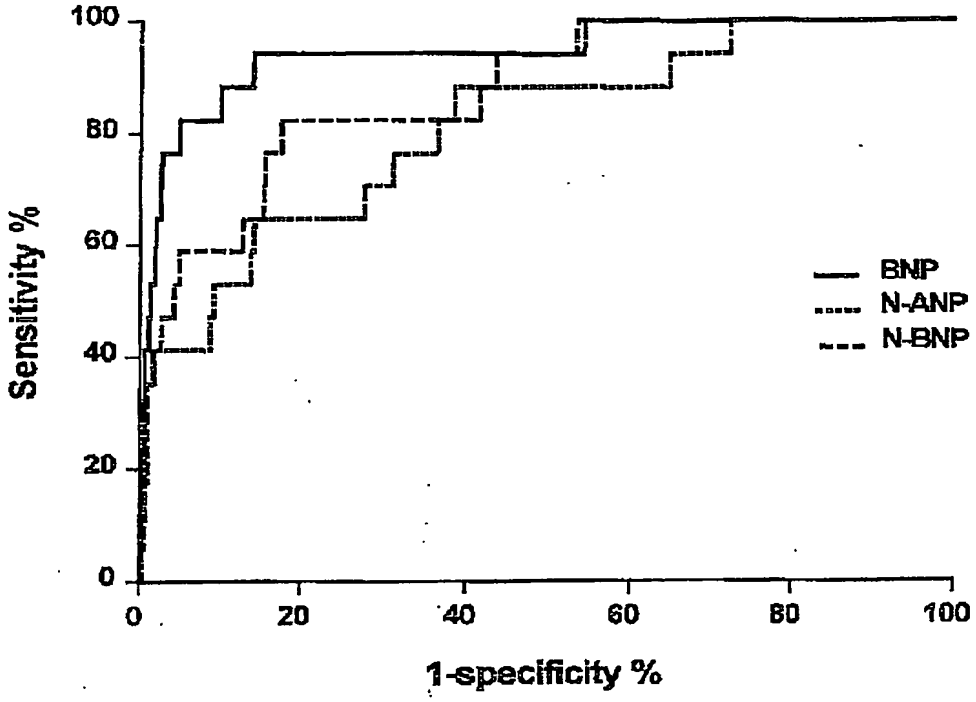


Figure 2.

ROC curves for BNP, full model (including $\log_{10}$ BNP, ECG and history of MI or angina) and minimal model (including $\log_{10}$ BNP level and ECG) in diagnosis of heart failure (as defined by a LVWMI score ≥ 2.

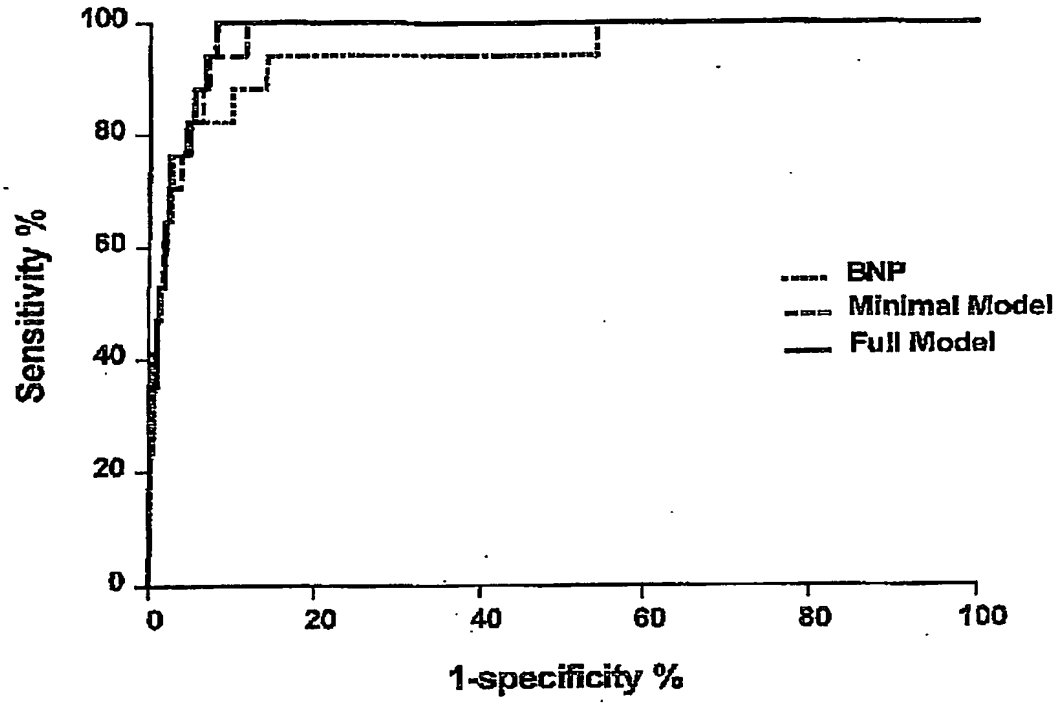
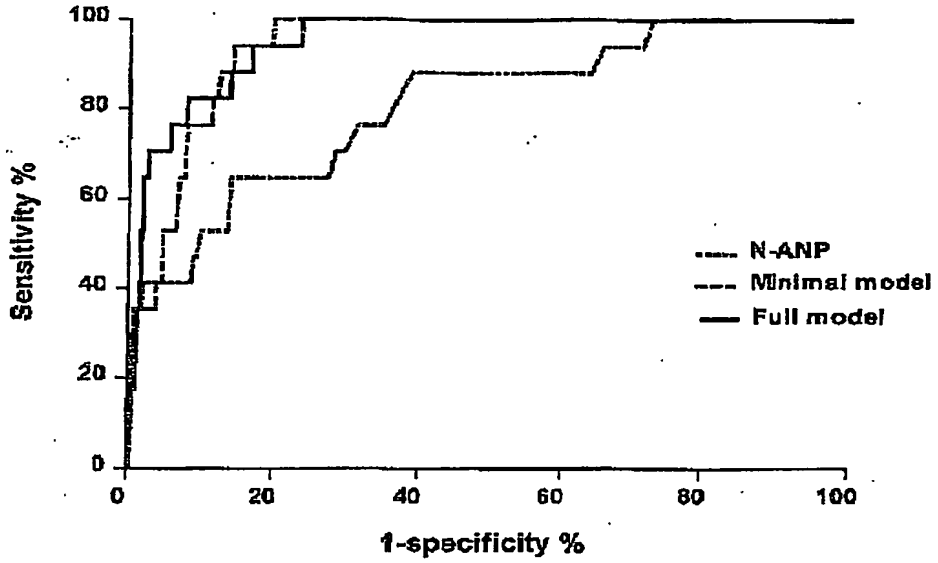


Figure 3.

ROC curves for N-ANP and N-BNP, their respective full models (including $\log_{10}$ peptide level, ECG and history of MI or angina) and minimal model (including $\log_{10}$ peptide level and ECG) in diagnosis of heart failure (as defined by a LVWMI score ≥ 2.



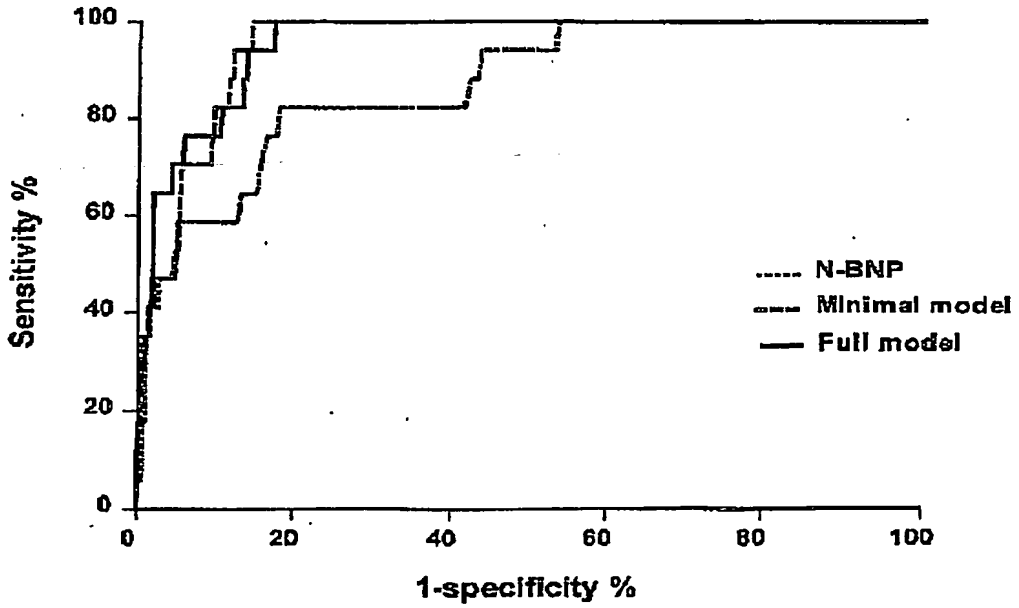


Figure 4.

ROC curves for BNP and N-BNP, their respective full models (including peptide level ranked as a percentile, ECG and history of MI or angina) and minimal model (including peptide level ranked as a percentile and ECG) in diagnosis of heart failure (as defined by a LVWMI score ≥ 2.